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Evidence for Preferential Mismatch Repair of Lagging Strand DNA Replication Errors in Yeast

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Summary

Duplex DNA is replicated in the 5′-3′ direction by coordinated copying of leading and lagging strand templates with somewhat different proteins and mechanics, providing the potential for differences in the fidelity of replication of the two strands. We previously showed that in *Saccharomyces cerevisiae*, active replication origins establish a strand bias in the rate of base substitutions resulting from replication of unrepaired 8-oxo-guanine (GO) in DNA [1]. Lower mutagenesis was associated with replicating lagging strand templates. Here, we test the hypothesis that this bias is due to more efficient repair of lagging stand mismatches by measuring mutation rates in *ogg1* strains with a reporter allele in two orientations at loci on opposite sides of a replication origin on chromosome III. We compare a MMR-proficient strain to strains deleted for the MMR genes *MSH2*, *MSH6*, *MLH1*, or *EXOI*. Loss of MMR reduces the strand bias by preferentially increasing mutagenesis for lagging strand replication. We conclude that GO-A mismatches generated during lagging strand replication are more efficiently repaired. This is consistent with the hypothesis that 5′ ends of Okazaki fragments and PCNA, present at high density during lagging strand replication, are used as strand discrimination signals for mismatch repair in vivo.

Results and Discussion

Lower mutagenesis associated with the lagging strand replication machinery [1] may result from differences in DNA polymerase nucleotide selectivity, exonucleolytic proofreading, or DNA mismatch repair (MMR). To test the latter of these possibilities, we took advantage of the fact that yeast *Saccharomyces cerevisiae* removes adenine incorporated opposite template GO by using the DNA mismatch repair system that corrects undamaged base-base mismatches generated during DNA replication [2, 3]. MMR of base-base mismatches [4–7] is initiated when a heterodimer of Msh2-Msh6 binds to a mismatch. Both yeast and human Msh2-Msh6 have been demonstrated to bind to a GO-A mismatch [2, 8]. Msh2-Msh6 binding is followed by several steps that are coordinated by a heterodimer of Mlh1-Pms1. Among these steps is excision of the error by exonucleases, at least one of which is the 5′-3′ exonuclease, Exo I [9, 10]. Excision is followed by correct DNA synthesis and then ligation. Although the signal that directs MMR to the newly synthesized strand in vivo is unknown, excision of the error in extracts of mammalian cells can be initiated at a nick located within a few hundred base pairs either 5′ or 3′ of the mismatch [11, 12]. This bidirectional excision capacity implies that both the 5′ and 3′ ends of DNA chains might act as strand discrimination signals in vivo. Because the lagging strand template is replicated discontinuously as Okazaki fragments of 150–250 bases, mismatches arising during lagging strand replication should have a 5′ terminus no further away than 250 bases (Figure 1). In contrast, continuous replication by the leading strand machinery is expected to generate a nascent strand in which the 5′ end at the origin is either sealed or at least far away from the majority of mismatches that are generated (Figure 1). In addition, more PCNA is needed to initially synthesize and then to process the 5′ ends of the many Okazaki fragments on the lagging strand than is needed for continuous leading strand replication. This may be relevant because PCNA participates in an early step or steps in MMR that precedes excision [13], and these steps likely involve the search for and use of the strand discrimination signal. For these reasons, we hypothesized that the strand bias for GO-induced mutagenesis in mismatch repair-proficient yeast [1] might result from more efficient repair of GO-A mismatches generated during lagging strand replication due to a higher density of strand discrimination signals.

To test this hypothesis, we first measured the reversion rate of the *ura3-29* reporter allele in the wild-type yeast strain 8C-YUNI101 [14] and its isogenic derivatives defective in *OGG1*, *MSH6*, or both. The reversion rate was higher in an *ogg1* strain than in a wild-type strain; it was higher by 22-fold overall and by 60-fold when considering the G-C to T-A transversions characteristic of replication of unrepaired GO (Table 1). Disruption of *MSH6* resulted in a 6-fold increase in the rate of G-C to T-A substitutions, while the reversion rate in the double *ogg1 msh6* mutant was elevated by 200-fold. This synergy indicates that GO-A mispairs arising during replication of the *ura3-29* allele are subject to MMR, as demonstrated in an earlier study at the *CAN1* locus [2]. We then extended this approach to four sets of yeast strains that contained the *ura3-29* reporter allele in either of two orientations and at either of two loci. These loci, designated *near306* and *agp1*, were adjacent to but on opposite sides of the frequently used early replication origin *ARS306* on yeast chromosome III (Figure 1). Each set compared a strain that was wild-type for MMR genes to four others that were deleted for either *MSH2*, *MSH6*, *MLH1*, or *EXOI*, genes whose products participate in the MMR steps mentioned above [4, 6, 7].

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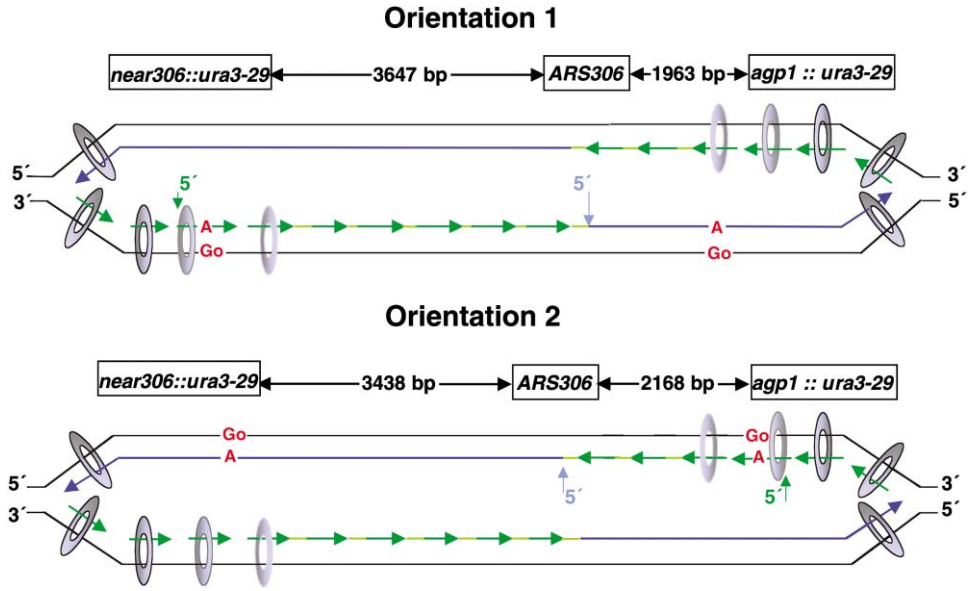

Figure 1. Schematic Representation of Replication Forks Emerging from *ARS306*

The location of *ARS306* and the two genetic loci used in this study are depicted, along with distances in base pairs from borders of *ARS306* to the site of the *ura3-29* mutation (not drawn to scale). The GO-A mismatches responsible for the G-C to T-A transversions in the *ogg1* strains are shown in red; GO in the template strand is at either the *near306* or *agp1* locus. For convenience, they are shown here as being present in the same molecule, but, in reality, they are present in either location in separate yeast strains. The nacsent strand generated by the leading strand replication machinery is blue, and the nacsent strand generated by the lagging strand replication machinery is green. The lighter green segments of the lagging strand represent the sequences involved in the formation and processing of 5′ flaps. The rings depict topologically bound PCNA molecules, which are suggested to be present at higher density during lagging strand replication. The lagging strand PCNA molecules closer to the origin are colored lighter gray to indicate that, after performing their roles in processing Okazaki fragments, they eventually dissociate or are unloaded by the RFC complex.

As shown previously [1] and again here (Table 2), the rate of GO-induced G→T substitutions in the *ogg1* strain that is MMR proficient depends on the orientation of the *ura3-29* gene and its location relative to *ARS306*. Opposite orientations of the *ura3-29* reporter gene have differences in mutation rates of 4- to 6-fold at both *near306* and *agp1* (Table 2 and gray bars in Figure 2). At both loci, the strand biases reflect lower error rates during lagging strand replication (as depicted in Figure 1). As expected based on an earlier study [2], *ogg1*-dependent mutagenesis was several-fold higher in all four MMR-defective strains examined (Table 2). Importantly, loss of MMR had a more substantial effect for GO-A mismatches generated during lagging strand replication. As an example, more than 80% of lagging strand (i.e., OR1) GO-A mismatches were corrected by Msh6-dependent repair at *near306*. In contrast, only 20% of leading strand (i.e., OR2) GO-A mismatches were corrected by Msh6-dependent repair at this same location. Effects of a similar magnitude were consistently observed for all four MMR-defective alleles (Table 2; Figure 2, black bars). These differential effects on mutagenesis reduce the orientation-dependent biases in the MMR-defective strains (Figure 2, black bars). For example, the 6-fold lower mutation rate in orientation one versus orientation two at *near306* in the mismatch repair-proficient strain is reduced to only a 1.3-fold bias in the *msh6* strain. A reduction in strand bias is also observed when the *ura3-29* is located on the other side of *ARS306* (at *agp1*, Table 2; Figure 2). In both cases, loss of Msh6 function preferentially increases mutagenesis associated with lagging strand replication. This implies that,

Table 1. The *ura3-29* Reversion Rates in Wild-Type and the *ogg1* and *msh6* Strains

Strain	Reversion Rate × 10^{-8} (95% Confidence Limits)	Relative Reversion Rate	C→A/Total	Relative Rate of C→A Transversions
Wild-type	1.3 (1.1–2.1)	1	6/16	1
ogg1	28 (22–37)	22	16/16	57
msh6	3.9 (3.1–5.5)	3	12/15	6.4
ogg1 msh6	94 (84–120)	72	16/16	193

As controls, mutation rates were measured for three other reporter genes (see the Experimental Procedures for references and Table S1).

Table 2. Rates of the *ura3-29* Reversions in Two Orientations in the *ogg1* and *ogg1* Mismatch Repair-Deficient Strains

MMR Gene Status	Chromosome III Location of the *ura3-29* Allele	*ura3-29* Orientation	Mutation Rate × 10^{-7}	
			Median	95% Confidence Limits
Wild-type	*near306*	OR1	1.3	1.7–2.3
		OR2	7.7	5.8–12
	agp1	OR1	4.0	3.7–6.6
		OR2	1.0	0.9–1.8
Δ*msh2*	*near306*	OR1	9.7	5.6–14
		OR2	13.0	7.3–17
	agp1	OR1	14.0	11–23
		OR2	8.8	7.1–10
Δ*msh6*	*near306*	OR1	7.6	4.1–11
		OR2	9.7	5.8–14
	agp1	OR1	9.0	6.7–16
		OR2	4.5	4.5–5.6
Δ*mlh1*	*near306*	OR1	13.0	11–20
		OR2	20.0	15–24
	agp1	OR1	12.0	10–24
		OR2	7.3	5.0–13
Δ*exo1*	*near306*	OR1	5.1	3.9–5.5
		OR2	10.0	8.6–12

As controls, mutation rates were measured for three other reporter genes located elsewhere in the genome. In pairwise comparisons of the OR1 and OR2 strains shown above, the mutation rates at those loci were not significantly different (see Table S2). This is consistent with the fact that those reporter genes maintained a constant sequence orientation relative to flanking origins and supports the interpretation that the strand biases shown above are significant and due to the relative location of the active flanking replication origin.

under normal circumstances, GO-A mismatches generated during lagging strand replication are more efficiently repaired than are GO-A mismatches generated by the leading strand replication machinery. The hypothesis that the reduced strand bias is due to loss of the general MMR pathway is supported by the fact that deletion of any of four different MMR genes had similar effects.

Because MMR efficiency can be affected by sequences flanking a mismatch [15, 16], we determined if loss of the strand bias for mutagenesis is also seen for mismatches at locations other than *ura3-29*. To do this, we determined Ura$^+$ to Ura$^-$ mutation rates in the *ogg1* and mismatch repair-defective *ogg1 msh6* strains and then sequenced the *URA3* gene in 152 independent *ura3* mutants to monitor strand biases for G-C to T-A transversions characteristic of GO-dependent mutagenesis. In the MMR-proficient (*MSH6*$^+$) strain (Table 3), the overall average rate of C-G to A-T events that reflect lagging strand errors in orientation 1 at *near306* (Figure 1) was 7.8-fold lower than the average rate of G-C to T-A events that reflect leading strand errors (Figure 1). In the *msh6* strain, this strand bias was reduced to 1.9-fold (Table 3). This result is similar to the *ura3-29* reversion data (Table 2) and further supports the hypothesis that GO-A mismatches generated during lagging strand replication are more efficiently repaired than are GO-A mismatches generated during leading strand replication.

More efficient MMR of lagging strand replication errors is consistent with the use of 5′ ends of Okazaki fragments as strand discrimination signals in vivo. During lagging strand replication, 5′ ends are present at higher density and would be closer to most replication errors than would the 5′ end of a continuously replicated leading strand (Figure 1). In addition, MMR proteins' interaction with the lagging strand replication machinery may be more efficient due to a higher density of PCNA on the lagging strand. PCNA participates in processing the 5′ ends of Okazaki fragments, i.e., sealing nicks and completing lagging strand replication, through it ability to interact with and stimulate the flap endonuclease FEN1 [17] and DNA ligase I [18]. PCNA also participates in the early steps of MMR [13] when strand discrimina-

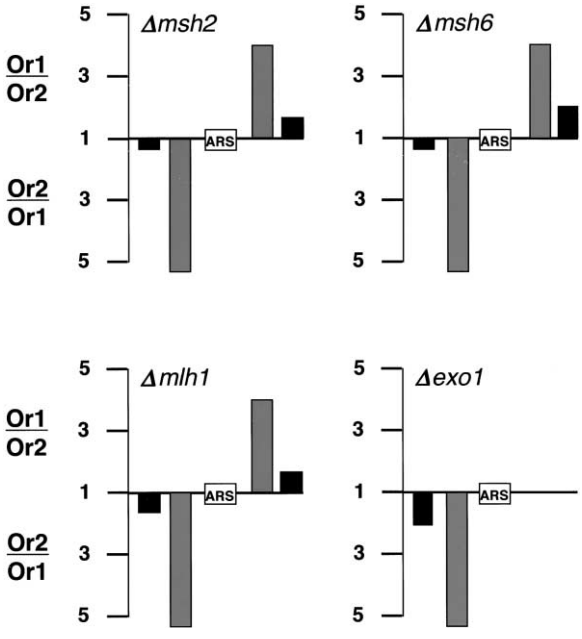

Figure 2. Orientation-Dependent Biases in Mutation Rates at Two Loci in MMR-Proficient and -Deficient Yeast Strains

The location of *ARS306* is indicated by ARS. The gray bars represent the strand bias in the MMR-proficient *ogg1* strain calculated from the data in Table 1. The black bars are the biases calculated for parallel mutation rate determinations in the MMR-defective strains listed.

Table 3. Forward FOA^r Mutation Rates, Summary of Sequencing Data, and Quantification of Biases

			ura3 Mutants Sequenced			Mutation Rate ($\times 10^{-7}$)		
Strain	Orientation	Mutation Rate ($\times 10^{-7}$)	Total	G→T	C→A	G→T	C→A	Ratio of G→T to C→A
MSH6	OR1	5.4	65	39	5	3.2	0.41	7.8
Δ*msh6*	OR1	46	87	39	21	21	11	1.9

The analyses were performed as described in the Experimental Procedures.

tion is required; it physically interacts with MLH1, MSH6, and MSH3 [13, 19–23], and it colocalizes with MSH3 and MSH6 in replication foci [22]. We previously suggested [13] that PCNA physically links the replication and MMR machinery and allows DNA ends to serve as strand discrimination signals. These ends are obvious entry points for excision of the nascent strand containing the error, followed by resynthesis of correct DNA. Recent biochemical data on MSH2-MSH6 interactions with PCNA are consistent with this hypothesis and led to the suggestions that MSH2-MSH6 interacts with PCNA when it is present on newly replicated DNA and that MSH2-MSH6 is transferred from PCNA to mispaired bases [23]. Hypothetically, a MMR model involving the 5′ ends of Okazaki fragments and a sliding clamp could also be relevant to prokaryotes. For example, the *E. coli* β sliding clamp has been shown to interact with MutS and with DNA ligase [24].

It is also theoretically possible that GO-A mismatches exist in multiple conformations that differ in their MMR sensitivity. For example, once adenine is incorporated opposite GO in a *syn* conformation (see [25]), subsequent rotation to an *anti* conformation might reduce MMR efficiency. The precedent for two, differentially utilized, *anti-syn* conformers of a modified guanine comes from an in vitro study of the efficiency of replication of template DNA containing O^6-methylguanine [26]. In the present study, this idea implies that such rotation would be more likely for leading strand replication GO-A mismatches, a lower proportion of which are subject to MMR (Table 2). The observation of higher MMR efficiency for lagging strand errors does not exclude the fact that a mutational strand bias during DNA replication could also result from differences in DNA polymerase selectivity or proofreading. In fact, a small but statistically significant (as determined by nonparametric criterion, see [1]) mutational bias persists for all *ogg1* yeast strains that are MMR deficient (see Figure 2; Table 2). This bias is consistent with the strand bias reported for replication errors in MMR-defective *E. coli* strains [27]. Given the hypothesis of more efficient MMR of lagging strand replication errors, it will be interesting to establish methods to determine if MMR defects have strand-specific influences on replication errors involving undamaged base-base mismatches or frameshift intermediates [28, 29]. If so, then variations in microsatellite instability or base substitution mutagenesis due to loss of MMR may depend not only on the nucleotide composition of the target gene, but also on its location relative to active replication origins.

Experimental Procedures

We used yeast strain 8C-YUNI101 [14] and its isogenic derivatives defective in *OGG1*, *MSH6*, or both. Forward mutation and nonsense and frameshift reversion rates in these strains are in agreement with the literature [2] (Table S1). We used *ogg1* derivatives of yeast strain Δl(-2)l-7B-YUNI300 (*MAT*a *CAN1 his7-2 leu2-*Δ*::kan*MX *ura3-*Δ *trp1-289 ade2-1 lys2-*Δ*GG2899-2900*) with integrations of the *LEU2-ura3-29* cassette with two orientations of the *ura3-29* allele into a given location in chromosome III [1]. Mismatch repair-deficient derivatives of these strains were generated by targeted gene disruption via transformation with PCR fragments carrying a selectable *TRP1* gene flanked by short sequence homology to the target gene. The plasmid pRS304 was used as a template for such PCR [30]. The primer pairs used were: disruption of *MSH2*: 5′-CTCCACTAGGCCAGAGCTAAAA TTCTCTGATGTATCAGAGGAGAGCAGAGCAGATTGTACTGAGAG TGCACC-3′ and 5′-CCTTCACTTTTCTAATCCACTCTTTCAGTAAAG CCTTCAAACGAACGCATCTGTGCGGTATTTCACACCGC-3′; disruption of *MSH6*: 5′-CCCAGCTACCCCTAAAACTTCTAAGACTGCA CACTTCGAAAATGGCAGAG CAGATTGTACTGAGAGTGCACC-3′ and 5′-AGACCCCTTCACCAGAACCTAATTTTGTATTCTTCAGTCCA TCTCCGCATCTGTGCGGTATTTCACACCGC-3′; disruption of *MLH1*: 5′-ATGTCTCTCAGAATAAAAGCAC TTGATGCATCAGTGGT TAACAAAACAGAGCAGATTGTACTGAGAGTGCACC-3′ and 5′-TGG AAGGTTGG CTATTTCCACGACATCCTTGAGAATGTGTCTAGGGG CGCATCTGTGCGGTATTTCACACCGC-3′; disruption of *EXO1*: 5′-ATG GGTATCCAAGGTCTTCTTCCTCAGTTAAAGCCCATACCAGAGCAG ATTGTAC TGAGAGTGCACC-3′ and 5′-TTGGGAAAGCAAGGAGA TAGATCTGACTGCCGGCCGAGCCGCATCTGTGCGGTATTTCAC ACCGC-3′.

To obtain strains for the forward mutation tests, spontaneous Ura$^+$ revertants from the *ogg1* and the *ogg1 msh6* strains with *ura3-29* in orientation 1 at the *near306* locus were selected and sequenced. All were pseudo wild-type; a change of codon TCT to TAT at the site of the *ura3-29*, which is characteristic for the *ogg1* strain, occurred in all strains (see [1]). These revertants were used for the mutation rate determinations in Table 2 and for obtaining the independent Ura$^-$ mutants for sequencing of the *URA3* forward mutations. DNA was obtained by PCR amplification of the whole *URA3* gene directly from yeast cells, and both DNA strands were sequenced by using a set of four sequencing primers and an ABI 3100 Genetic Analyzer. Mutation rates were determined as described earlier [1, 14]. To calculate a percentage (F%) of GO-A mismatches corrected, we used the formula: $F\% = (1 - [f_{wt}/f_{mmr}])*100$, where f_w and f_{mmr} are C→A transversion rates in the *ogg1* and the *ogg1* MMR$^-$ strains, respectively.

Supplemental Data

Supplemental Data including mutation rate measurements for several additional markers are available at http://images.cellpress.com/supmat/supmatin.htm.

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